

Physiological Behavior of Grimes Golden Apples in Storage

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POMOLOGY SUBSECTION



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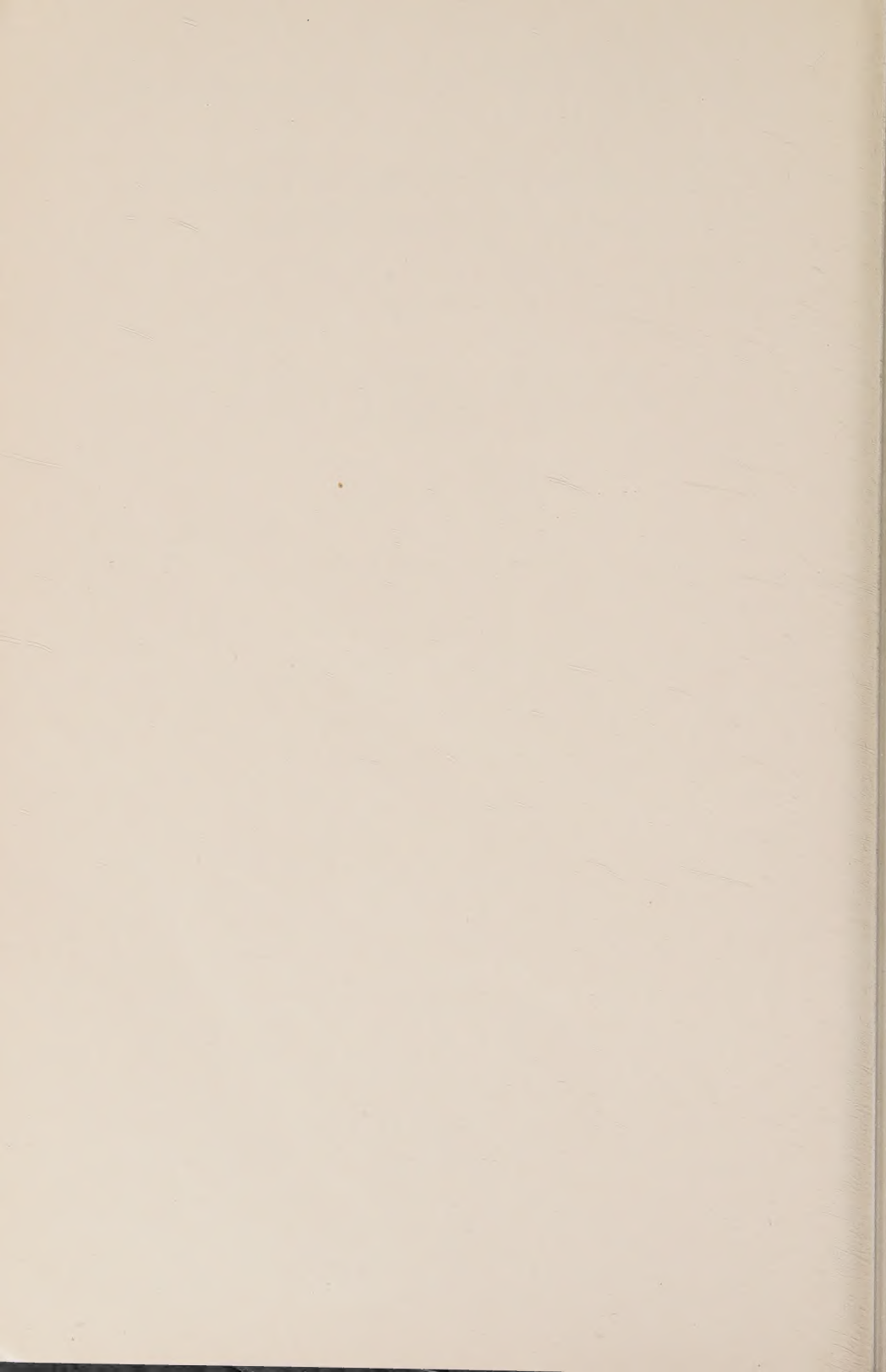
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SUMMARY—PART I

1. Respiration determinations were made on Grimes Golden apples at intervals throughout two storage seasons at storage temperatures of 60°, 50°, 36° and 30° F.

2. Temperature was the outstanding factor influencing respiratory intensity. A close correlation between temperature and respiration was found to exist when the fruit was subjected to alternating temperatures. When the temperatures of Grimes apples were alternated three different times during the storage period (at approximately monthly intervals) from 50° to 30° F. and vice versa, the respiration rate was lowered or increased, depending upon whether the change was from the higher temperature to the lower or from the lower to the higher. The rate, however, was neither stimulated nor depressed further (after the fruit samples had reached temperature equilibriums of 50° and 30° F.) than the respiration rate of fruit held constantly at the two temperatures.

3. It required about 60 hours for fruit at 50° F. to reach 30° F. upon being transferred to the latter temperature, and vice versa.

4. Respiratory activity of apples just at the time of placement in storage serves as an index to the storage capacity of fruit, particularly with respect to soggy breakdown. Fruit picked and held at a moderately high temperature soon reached a high rate of respiration. When transferred from a stage of high activity to a lower temperature where retarded activity resulted, there was a disturbance within the tissue subsequently shown by soggy breakdown.

5. Soggy breakdown developed with deferred storage when Grimes at 50° were transferred to a temperature of 30° F., providing the transfer came during the period of high respiratory activity; otherwise no breakdown developed. The highest percentage of soggy breakdown occurred when Grimes were transferred while at the peak of respiration at 50° to 30° F.

6. The fruit's life was prolonged by placing it in storage immediately after being picked. In the lower temperatures respiratory activity was reduced to a minimum.

7. The curve depicting the respiratory activity of the Grimes apple throughout its storage period at 50° F. indicated four distinct stages of intensity. These were (1) a short period of accelerated ascent; (2) a short period of rapid descent; (3) a longer period of gradual descent; (4) a period of senescence, which gave rise to a slightly increased rate of respiration, because of such complications as mealy breakdown and scald.

8. At a temperature of 50° F. apples from high nitrogen treatments respired consistently more than did the check lots at this temperature. At 36° or 30° F. temperature was the controlling factor in respiration and previous nutrient and environmental conditions had little effect.

9. A higher percentage of soggy breakdown developed with deferred storage fruit from the high nitrogen treatment than from the check lots of apples.

Physiological Behavior of Grimes Golden Apples in Storage¹

BY PAUL L. HARDING²

Part I. Respiratory Intensity

Practically all storage investigators have emphasized the importance of storing apples immediately after they are picked. The reason for this practice is better understood when it is realized that with a rise in temperature there is a corresponding increase in metabolic activity which may remain at a relatively high level for a considerable period. When this occurs apples ripen very rapidly. Placing fruit under temperatures slightly above freezing, however, slows down activity to a minimum, and the life of the fruit is greatly prolonged. A picture, then, of respiratory activity going on within the tissues of the apple, indicates how fast or how slowly metabolism is proceeding. Thus, in order to slow down activity within the fruit it should be placed in storage as soon as possible after it is picked. Temperatures of 50° to 80° F., which are common at picking time, greatly increase respiration, and maximum activity is reached within a very short time.

Holding fruit in the orchard or warehouse for several days or weeks before it is placed in storage may result in internal metabolic disturbances. Transferring fruit from high temperatures with accompanying high respiratory activity to lower storage temperatures places it under conditions in which a minimum of change takes place. Changes in respiratory intensity under these extremes of temperature may be readily determined by measurement of the rate of CO₂ evolution from the fruit. It is recognized that this method of measuring respiratory intensity does not give a complete analysis of respiration processes; nevertheless, measurement of CO₂ evolution is generally considered a reliable index of metabolic changes. Just how these changes affect the apple is difficult to answer. It is known, however, that changes which accompany wide temperature fluctuations may result in certain functional disorders. (45) (46).

The purpose of this investigation is to determine the role

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which respiration, catalase and oxidase play in the development of these disorders, especially in the development of soggy breakdown, and incidentally to determine any inter-relationship between respiratory intensity and catalase and oxidase activity.

HISTORICAL

Respiration of plants and animals has attracted the attention of scientific workers from a very early date. Results of these experiments and the general subject of respiration have been reviewed extensively by Bigelow and Gore (4), Hill (26), Palladin (44) and Kostychev (33). The subject of respiration is so broad that only the work specifically dealing with respiration of apples under various temperature conditions will be reviewed.

Extensive work by Bigelow and Gore (4) and Gore (15) showed that the rate of respiration increased with the rise of temperature according to Van't Hoff's law. Morse (41) measured the amount of carbon dioxide given off from apples stored at 32°, 50° and 68° F. and showed that lower temperatures retarded respiratory activity and thus prolonged the fruit's life. Magness et al. (39) showed that there was a very close agreement between the rates of respiration and the softening of Grimes Golden apples. Kidd and West (30) pointed out that it might be possible to predict the keeping quality of apples from observations of certain chemical properties and a knowledge of their respiratory activity. Kidd and West (28) (31) agreed with Magness et al. (39) and Burroughs (7) that long life of apples was related to low respiratory activity. Harding (18) pointed out that the rate of respiration increased with the development and maturity of the fruit under a uniform temperature.

Hill (26) and others (5), (27), (49), (28), (38) have studied fruit behavior under aerobic and anaerobic conditions. These investigations indicated that injury was due to insufficient oxygen and also possibly to the accumulation of carbon dioxide. Magness (36), Magness and Burroughs (37) and Magness and Diehl (38) determined the ratio of carbon dioxide to oxygen within the intercellular spaces of apple tissue and the effect of temperature on this relationship.

Magness and Burroughs (37) showed that apples held at storage temperatures of 32° and 36° F. respired at a very constant rate throughout the storage season. Burroughs (8) and Magness and Diehl (38) studied respiratory activity of apples through the ripening period and the effect of alternating temperatures, wounding, coating the fruit with oil and paraffin, and their effect on the subsequent respiration rate at 68.5° F.

In studies relative to the effect of freezing on apple tissue Carriek (9) found that lowering the temperature from 0° C. to -8° C. reduced carbon dioxide evolution 90 times. He also found (10) that by freezing apples a few hours and then measuring the respiration rate at 0° C., the amount of carbon dioxide was increased 85 percent. Some stimulatory effect was noticeable a month later.

Kidd and West (28) presented curves showing the variation in the respiratory activity in storage of single apples from the same sample. Their work also included curves showing intensity of respiration of apples from three soil-types, viz., fen, gravel and silt. Apples from fenland soil respired most rapidly, had a shorter storage life, contained the highest percentages of nitrogen and were low in sucrose. Apples from gravel soil entered storage more advanced in maturity, respired less than apples from fen soil, had the shortest storage life and were low in both nitrogen and sucrose. Apples from silt soil respired least rapidly, had the longest commercial storage life, were low in nitrogen and high in sucrose.

Harding (18) has shown that the respiration rate of Grimes Golden apples increased rapidly at a temperature of 50° F. and reached its highest intensity 4 to 5 weeks after picking. This period of highest respiratory activity was immediately followed by a period of sharp decline and still later by a period of slower activity. Apples stored while in the period of highest respiratory activity subsequently developed the largest proportion of soggy breakdown.

Kidd and West (29) have recently called attention to the similarity of the respiration curve's form of Grimes Golden apples as noted by Harding (18) to that of the English variety, Bramley's Seedling, at the same temperature. They (29) have also recognized that Bramley's Seedling is most susceptible to the low-temperature type of breakdown when it is stored while in the stage of highest respiratory intensity.

METHODS AND APPARATUS FOR CARBON DIOXIDE DETERMINATION

Grimes Golden apples for these investigations were obtained largely from the Apple Grove Orchards, Mitchellville, Iowa. The soil had previously received applications of sodium nitrate at the rate of 5 pounds per tree in the springs of 1927 and 1928. In order to determine more accurately the keeping quality of fruit from trees treated with nitrogenous fertilizers, the application of nitrate of soda was increased in 1929 to 10 pounds per tree. Apples used for respiration investigations in 1928-29 were from trees receiving the two applications of 5 pounds per tree

per year in 1927 and 1928. In 1929-30 apples used in the respiration experiments were from trees which received 10 pounds per tree in 1929, in addition to 5 pounds per tree for 1927 and 1928. The latter treatment will be hereafter designated as "high nitrogen." The treatment in which the trees received 5 pounds of nitrate of soda in the years 1927 and 1928 will hereafter be designated as "nitrate 5-5;" whereas the treatment in which the trees received 5 pounds of the nitrate fertilizer in the years 1927 and 1928, and 10 pounds in 1929, will be designated as "nitrate 5-5-10."

For the check apples Grimes were obtained from a nearby orchard owned by Cyrus Harvey. The fruit was smaller, and the trees showed typical characteristics of trees suffering from nitrogen deficiency. Trees on the nitrated plot, in contrast, were vigorous, had large leaves and the fruit graded large.

Grimes from these orchards were picked on the same day. Within 12 hours from the time of picking they were graded, packed, transported to Ames, weighed, placed in large respiration chambers and subjected to the desired temperatures.

Reference has been made to the investigations of Kidd and West (28) wherein a variation of respiratory activity was found to occur with single apples selected from the same samples. Maney, Harding and Plagge (40) recognized this variation of single apples due to differences of maturity. In order to reduce the high experimental error resulting from small samples, they designed a large pickle bottle respiratory chamber which was used throughout the following investigations.

The procedure followed in the use of the apparatus for the determination of carbon dioxide as a measure of respiration was exactly that described by Harding, Maney and Plagge (19).

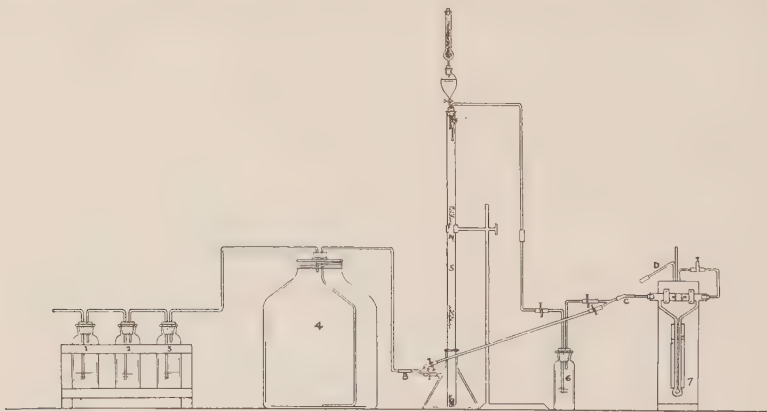


Fig. 1. Apparatus for the determination of carbon dioxide as a measure of respiration.

The apparatus is shown in fig. 1. Air was drawn through two wash bottles, 1 and 2, containing 50 percent potassium hydroxide. It was then bubbled through barium hydroxide, in bottle 3, as a check on the absence of carbon dioxide. Connections with the respiration chamber, 4, were made with copper tubing in such a way as to draw the carbon dioxide-laden-air from the bottom of the bottle. At the points B and C, "Y" tubes were placed in order that the air stream might be directed either through the absorption tower for the measurement of carbon dioxide or directly to the flow-meter in case continuous aspiration was desired without measuring the carbon dioxide. A simple manipulation of steel clamps permitted the air to pass through either system. This system made possible the removal of carbon dioxide which might have accumulated in the respiration chamber previous to a determination. It was possible to make connections with the absorption tower with only a momentary stoppage of the air-stream. Continuous aspiration was practically accomplished with no loss and very little accumulation of carbon dioxide in the respiration chamber.

Carbon dioxide was absorbed in a Truog absorption tower, 5 (50). Bottle 6 contained barium hydroxide and served to indicate whether carbon dioxide was passing off unabsorbed. A flow-meter, 7, was adapted to this apparatus in order to keep the rate of aspiration uniform and of a known velocity. The flow-meter was connected with the aspirator at the point "D." The reagents used in carrying out the titrations were N/5 solutions of standard hydrochloric acid and barium hydroxide, with phenolphthalein as an indicator.

In measuring carbon dioxide evolution at a given temperature it is of utmost importance that constant temperatures be maintained, since fluctuation in temperature soon influences respiratory activity. Temperature and relative humidity in the various storage rooms were kept very uniform.

EXPERIMENTAL

EFFECT OF ALTERNATE TEMPERATURES UPON CARBON DIOXIDE EVOLUTION

As a preliminary step in this investigation, in order to observe the effect of alternate temperatures upon carbon dioxide production, two jars of Grimes Golden apples were held at 50° F. until the experiment was initiated on Nov. 1. Then jar 1 was placed at 30° and jar 2 remained at 50°. Fruit in the respiration chambers was allowed to reach the temperature of the storage room before respiration determinations commenced. From table 1 it may be noted that 60 hours were necessary for fruit at 50° to reach a temperature of 30° or for fruit at 30°

TABLE 1. RELATION OF FRUIT TEMPERATURES TO RESPIRATORY INTENSITY.

No. of hours after transfer	Transferred from 50° to 30°F.						Transferred from 30° to 50°F.					
	Nov. 21		Dec. 15		Jan. 17		Nov. 21		Dec. 15		Jan. 17	
	Fruit temp. in F.	Mg. CO ₂ per kg. hr.	Fruit temp. in F.	Mg. CO ₂ per kg. hr.	Fruit temp. in F.	Mg. CO ₂ per kg. hr.	Fruit temp. in F.	Mg. CO ₂ per kg. hr.	Fruit temp. in F.	Mg. CO ₂ per kg. hr.	Fruit temp. in F.	Mg. CO ₂ per kg. hr.
0	50	11.35	50	9.50	50	7.22	30	3.64	30	3.00	30	3.06
2	46	8.93	46	8.94	46	7.15	34	3.70	34	3.14	35	3.22
6	40	6.67	40	7.22	40	5.86	38	5.16	40	3.46	40	3.38
12	36	5.51	36	5.53	36	4.71	42	6.46	42	4.50	43	5.91
24	35	4.35	34	4.47	34	3.62	46	10.78	46	6.33	46	6.97
48	32	3.51	31.5	3.39	32	3.47	49	12.60	48	8.07	48	7.44
60	30	3.51	30	3.25	30	3.15	50	12.84	50	8.15	50	8.00

to reach 50°. Temperature of the fruit was readily determined by placing a thermometer into an apple within each respiration chamber.

Three respiration determinations were made at intervals of one week. At the end of the third week the respiration chambers were interchanged. Jar 1 was transferred from 50° to 30° and jar 2 from 30° to 50°. Again the fruit was allowed to reach the given temperatures before three more determinations at intervals of one week were made. Such a transfer was made three times during the season and the experiment repeated dur-

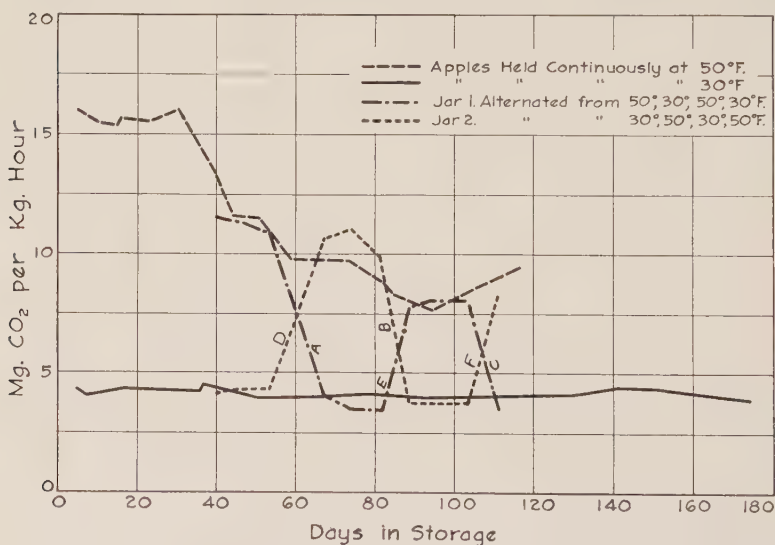


Fig. 2. The effect of alternate temperatures on the respiration rate of Grimes Golden apples.

ing the two seasons of 1928-29 and 1929-30 with practically the same results. The results are shown graphically in fig. 2. Regardless of the previous stimulatory effect upon the fruit subjected to a higher temperature, when returned to 30° the respiration rate was reduced to a minimum, and each time approximately the same minimum respiration rate was reached. Conversely, each time the fruit was replaced at 50°, the higher temperature increased the respiration rate. At 50°, however, the rate of respiration is more dependent upon respiratory reserves as well as temperature. A general lowering of respiration was observed as the season advanced. Therefore, at the higher temperature, the stage of development or maturity of the fruit expressed itself in its effect on the respiration rate. This condition has been previously emphasized by the author (18).

The controlling influence of temperature upon the respiratory activity of Grimes Golden is shown in fig. 2. Whenever the fruit temperature was 30° F. the respiration rate was at a minimum. The respiration rate was increased whenever the fruit was at the higher temperature of 50° F. The evolution of carbon dioxide of Grimes held continuously at 50° and 30° F., is shown. Samples of fruit held at 30° F. and 50° F. maintained independent rates of respiration; but when the fruit at 50° F. was shifted to a temperature of 30°, and vice versa, both samples of fruit then assumed approximately the respiratory intensity prevailing at the temperature to which they were submitted.

RESPIRATORY ACTIVITY OF APPLES AT INTERVALS DURING CHANGES OF TEMPERATURE

Apparatus and conditions in general were admirably suited to measure the respiration rate of the fruit at intervals during the period of its adaptation to the alternate temperatures. Flow-meters accurately governed the velocity and volume of aspiration and thus maintained the same rate of aspiration after the exchange of respiration chambers to the respective temperatures. This exchange of respiration chambers was accomplished within 2 minutes' time. Precautions were made to prevent contact with air laden with carbon dioxide; therefore the only excess accumulation of carbon dioxide was that accumulated during the short 2-minute interval of transfer. Two hours elapsed before the first determinations were made, to reduce to a minimum any excess carbon dioxide that might have accumulated during the transfer.

Table 1 shows the intervals at which the determinations were made, and the amounts of carbon dioxide evolved at the specific

fruit temperatures. The respiration jars were interchanged three times during the season: Nov. 21, Dec. 15 and Jan. 17. The fruit was the same as that used in the experiment of "Alternating Temperatures," and the dates just listed are periods when the respiration chambers were alternated from 50° to 30° F. represented in fig. 2 by sections A, B and C, and from 30° to 50° F. represented by sections D, E and F. The lines lettered A to F in fig. 3 refer to the sections of the corresponding letters in fig. 2. Figure 3 shows that the production of carbon dioxide was closely related to temperature of the fruit. The rate of carbon dioxide production diminished gradually as the temperature of the fruit lowered, as shown by curves A, B and C, and likewise increased with the temperature when the change was made from 30° to 50° as shown by curves D, E and F (fig. 3). The rate, however, was neither stimulated nor depressed further (after the fruit samples had reached temperature equilibriums of 50° and 30° F.) than the respiration rate of fruit held constantly at the two temperatures.

The amount of respiratory material available was evidently greater when the fruit temperature reached 50°, and this probably accounts for the differences in the amounts of carbon dioxide as shown by curves D, E and F. However, the general trend of respiratory activity shown by the latter curves was consistent. The amount of carbon dioxide evolved was thus dependent not only upon the fruit temperature but also to some

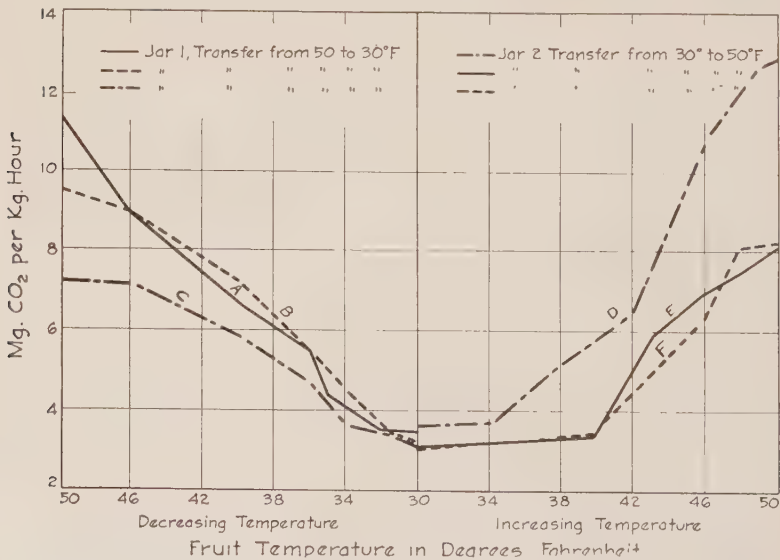


Fig. 3. The respiration rate of fruit at intervals during its change of temperature.

extent upon the age of the fruit which affected the amount of respiratory material.

EFFECT OF MATURITY ON RESPIRATION

In the early fall of 1928 respiration studies commenced three weeks previous to the time when Grimes were picked for commercial purposes. Measurements made on three different samples of Grimes Golden picked at periods one week apart indicated the respiration rate accompanying the development of the fruit and gave a more complete story of the fruit's metabolic activity. These pre-commercially picked apples were placed under aspiration at 50° F. for 60 hours before determinations were commenced.

Data in table 2 show that there was a gradual increase in carbon dioxide production with the fruit development. It will be shown later in table 6 and fig. 4, curve 2, that this increase continued for a time with commercially picked apples stored at a uniform temperature of 50° F. After a maximum respiration rate is reached, a period of rapid descent follows, which gradually decreases as the season advances.

TABLE 2. RATE OF RESPIRATION OF PRE-COMMERCIALY AND COMMERCIALY PICKED GRIMES FRUIT AT A STORAGE TEMPERATURE OF 50 F.

Date of determination	Number of apples	Wt. of fruit in grams	Mg. CO ₂ per Kg. hr.
Pre-commercial picking			
Lot 1			
Sept. 9	72	8,791	9.59
Sept. 10	72	8,791	9.08
Lot 2			
Sept. 16	70	8,672	10.19
Sept. 17	70	8,672	10.23
Lot 3			
Sept. 21	70	8,959	12.29
Sept. 22	70	8,959	12.08
Commercial picking			
Lot 4			
Sept. 26	64	8,846	14.38
Sept. 27	64	8,846	14.38

RESPIRATION EXPERIMENTS WITH GRIMES IN STORAGE DURING SEASON OF 1928-29

Pressure tests to determine maturity were made, but common commercial practices governed the date of commercial picking. Fruit for this investigation was obtained during the commercial harvest which started a few days previous to Sept. 25. With the exception of the one jar of apples held at 60° F. by means of a

TABLE 3. TREATMENTS TO WHICH THE SIX LOTS OF GRIMES GOLDEN APPLES OF THE COMMERCIAL PICKING WERE SUBJECTED. 1928-29.

Jar no.	Storage temp. F.	Treatment	Number of apples	Weight of fruitingms.
1	60	Immediate storage, water bath control of temperature	60	7,850
2	50	Immediate storage, cold storage	64	8,846
3	36	Immediate storage, cold storage	65	8,756
4	36	3 weeks delayed at 50°F. before storage at 36°F.	68	8,860
5	30	Immediate storage, cold storage	70	8,659
6	30	3 weeks delayed at 50°F. before storage at 30°F.	65	8,608

water bath, the storage temperatures selected for the respiration studies were 50°, 36° and 30° F. (Table 3.) One lot was placed immediately in each of the above four temperatures. Two lots were held at 50° F. for 3 weeks, and then one of them was placed in cold storage at 30° and the other at 36° F. Respiration determinations were made on all six lots of apples at weekly intervals throughout the storage season.

Figure 4 indicates that the respiratory rate of apples placed at 60° followed a curve similar in form to that of the fruit at 50° F. The maximum respiration rate of apples stored at 60° F., however, was reached about 3 weeks from the time of storage,

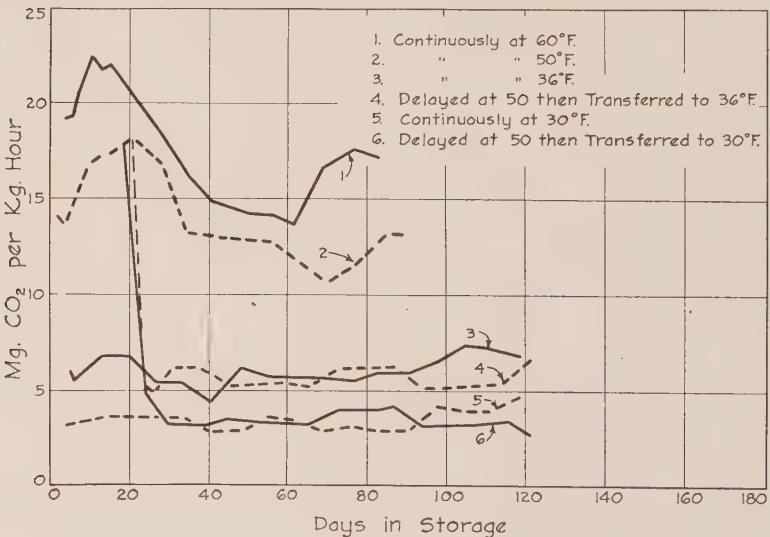


Fig. 4. Evolution of carbon dioxide from Grimes Golden apples under various controlled temperatures. Storage season 1928-29.

as compared with 4 to 5 weeks for the fruit stored at 50° F. Differences in storage temperatures created differences in maturity, which caused a variation in the amount of carbon dioxide evolved; consequently the quantity of CO₂ given off must also be considered from the standpoint of maturity as well as temperature.

When fruit is held in the orchard, packing shed or warehouse for days or weeks before being placed under storage temperatures, it may be transferred at a time of high respiratory activity to a temperature which, in 2½ days, cuts the rate of respiration to a minimum. Maximum soggy breakdown was observed by Plagge (46) when apples were delayed for 3 to 5 weeks at 50° F. and then transferred to 30° or 32° F. This breakdown was reduced to a minimum when the transfer was made to 36° F.

Apples which furnished the data shown in fig. 4 were samples obtained from the same lots used in studies reported by Plagge (46). It is shown in fig. 4 that the above transfer came at the peak of respiration. When Grimes were transferred during the period of their highest respiratory activity after 2 to 5 weeks' delay at 50° to 30° or 32° F., soggy breakdown resulted. When they were transferred later, or during a period of lower respiration, after 6 to 12 weeks' delay, no soggy breakdown was observed. Apples retained throughout storage at 50° F., which is more nearly the temperature of common storage houses in Iowa (during the first six weeks of storage) developed no soggy breakdown. Soggy breakdown appeared when the transfer occurred during high respiratory activity but did not develop when the transfer was made later during lower respiratory intensity.

The respiration rate of Grimes apples stored immediately at 30° and at 36° was more constant than that of the fruit delayed at 50° F. for 3 weeks before being placed at the lower temperatures. As the storage season advanced, there was a gradual increase in the respiration rate in the apples placed in storage temperatures of 30° or 36° F. In the delayed fruit there was an abrupt, although slight, rise in the rate of respiration some 70 days after picking. This increased rate continued for about 3 weeks. It was shortly after this abrupt increase in respiration that soggy breakdown was first observed within the respiration chambers.

On Jan. 25, after the apples had been in storage about 4 months, fruit in the respiration jars was examined for breakdown with the results shown in table 4. Other collateral storage lots (46) showed the following percentages of soggy breakdown: 3 weeks delayed storage at 50° before storage at 30° F., 60.3 percent; 3 weeks delayed storage at 50° before storage at 36° F., 8.7 percent.

TABLE 4. CONDITION OF GRIMES APPLES IN THE RESPIRATION CHAMBERS ON JAN. 25, 1929.

Jar no.	Storage temp. F.	Treatment	Condition of the fruit
1	60	Immediate storage, water bath control of temperatures	Respiration determinations extended until fruit had spoiled
2	50	Immediate storage, cold storage	Respiration determinations extended until fruit had spoiled
3	36	Immediate storage, cold storage	Fruit sound
4	36	3 weeks delayed at 50 F. before storage at 36°F.	28 percent soggy breakdown
5	30	Immediate storage, cold storage	Fruit sound
6	30	3 weeks delayed at 50°F. before storage at 30°F.	82 percent soggy breakdown

RESPIRATION EXPERIMENTS WITH GRIMES IN STORAGE DURING SEASON OF 1929-30

Grimes Golden apples for the respiration and storage investigations for 1929-30 were again obtained from the Apple Grove and Cyrus Harvey orchards, Mitchellville, Iowa. The fruit was taken from the same trees from which the apples were obtained for the experiments of 1928-29. In addition to the two previous applications of nitrate fertilizer (nitrate 5-5) the soil received a double application of 10 pounds of nitrate of soda (nitrate 5-5-10) per tree in the spring of 1929.

The date of commercial picking for 1929 was practically the same as that of the previous year. Again the apples for the respiration investigations were under storage temperatures and under aspiration within 12 hours from the time of picking. The storage temperatures were similar to those used in 1928-29, with one exception, which was the lot subjected to 60° F. The treatments to which the 10 lots were subjected are shown in table 5.

RELATION OF RESPIRATION TO TEMPERATURE, STORAGE CONDITIONS AND SOIL FERTILIZATION WITH NITRATES

Grimes from both the nitrate-treated trees and the check lots were immediately placed in storage temperatures of 50°, 36° and 30° F. Comparable lots of apples were retained at 50° F. for 3 weeks before being placed at the subsequent lower temperatures of 36° and 30° F. Respiration determinations were made at weekly or at 10-day intervals throughout the storage season on both the immediately stored fruit and the delayed lots. The respiration rate of Grimes stored continuously at 50° F. is shown in table 6 and fig. 5. The rate of respiration agreed closely with that of fruit of the previous storage year,

TABLE 5. TREATMENTS TO WHICH THE TEN LOTS OF APPLES OF THE COMMERCIAL PICKING WERE SUBJECTED. 1929-30.

Jar no.	Storage temp. F.	Treatment	Number of apples	Weight of fruitingms.
1	50	Immediate storage, cold storage. High nitrogen (nitrate 5-5-10)	72	7,800
2	50	Immediate storage, cold storage. Check (No nitrogenous fertilizer)	70	8,210
3	36	Immediate storage, cold storage. High nitrogen (nitrate 5-5-10)	74	8,052
4	36	Immediate storage, cold storage. Check (No nitrogenous fertilizer)	65	8,218
5	36	3 weeks delayed at 50°. before storage at 36°. High nitrogen (nitrate 5-5-10)	53	7,991
6	36	3 weeks delayed at 50°. before storage at 36°. Check (No nitrogen)	76	8,357
7	30	Immediate storage, cold storage 30°. High nitrogen (nitrate 5-5-10)	62	8,145
8	30	Immediate storage, cold storage 30°. Check (No nitrogenous fertilizer)	72	7,815
9	30	3 weeks delayed at 50° before storage at 30°. High nitrogen (nitrate 5-5-10)	74	8,127
10	30	3 weeks delayed at 50° before storage at 30°. Check (No nitrogen)	82	8,326

and it is observable that the curve may be sub-divided as indicated into four distinct stages.

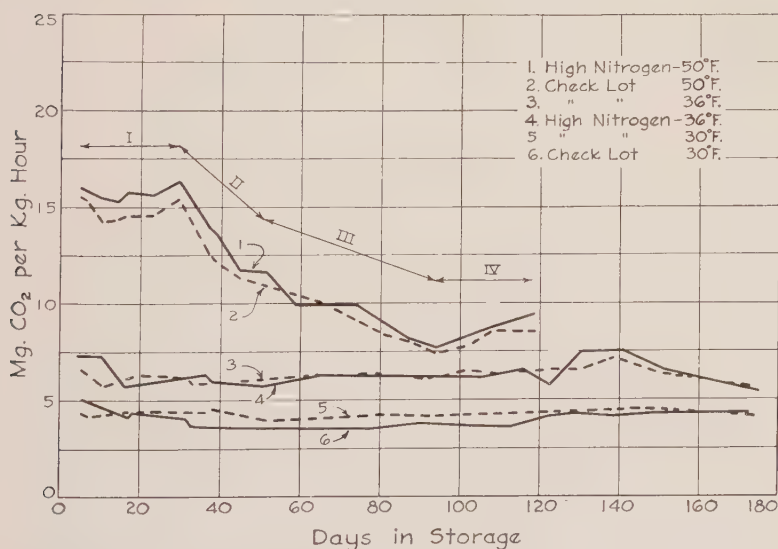


Fig. 5. Relation of the respiration rate of immediately-stored fruit to soil fertilization with nitrate. Stages I, II, III and IV of respiratory intensity. Season 1929-30.

TABLE 6. THE RATE OF RESPIRATION OF GRIMES GOLDEN APPLES WHEN STORED IMMEDIATELY AT 50° AND 60°F. RESULTS FOR SEASONS 1928-29 AND 1929-30.*
Mg. CO₂ per kg. per hr.

Date of determination	Days in storage	1928-29		1929-30	
		Nitrate 5-5		Nitrate 5-5-10	Check
		60°F.	50°F.	50°F.	50°F.
Sept. 26	1		14.38		
Sept. 28	3	19.18	13.63		
Sept. 29	4			16.05	15.53
Sept. 30	5	19.25	14.08		
Oct. 1	6		14.51	15.90	15.35
Oct. 2	7	20.70			
Oct. 3	8	21.22			
Oct. 5	10	22.55	16.94	15.50	14.10
Oct. 8	13	21.70	17.41		
Oct. 9	14	22.00	17.40	15.30	14.20
Oct. 11	16			15.70	14.55
Oct. 15	20	20.80			14.55
Oct. 16	21		18.10		
Oct. 18	23			15.65	14.60
Oct. 22	27	18.58			
Oct. 23	28		16.82		
Oct. 25	30			16.10	15.40
Oct. 29	34	16.30			
Oct. 30	35		13.29		
Nov. 1	37			13.95	12.30
Nov. 2	38			13.65	12.10
Nov. 5	41	14.92			
Nov. 6	42		13.06		
Nov. 8	44			11.65	11.30
Nov. 12	48	14.47			
Nov. 13	49		13.04		
Nov. 15	51			11.60	11.10
Nov. 19	55	14.36			
Nov. 20	56		12.94		
Nov. 22	58			9.89	10.20
Nov. 26	62	13.77			
Nov. 27	63		11.79		
Nov. 29	65				10.00
Dec. 3	69	16.81			
Dec. 4	70		10.64		
Dec. 7	73			9.89	
Dec. 10	76	17.60			
Dec. 11	77		11.55		
Dec. 13	79				8.41
Dec. 14	80			9.04	
Dec. 17	83	17.26			
Dec. 18	84		13.43		
Dec. 20	86			8.15	
Dec. 21	87				8.02
Dec. 25	91		13.35		
Dec. 28	94			7.72	7.52
Jan. 5	102			8.48	7.87
Jan. 10	107			8.82	8.63
Jan. 20	117			9.50	8.52

*Apples picked Sept. 25 in 1928; Sept. 26 in 1929.

STORAGE TEMPERATURES AND RESPIRATORY INTENSITY

Immediately-stored fruit at 36° and 30° F. respired at a very constant rate throughout storage, the rate being closely related to storage temperature. The respiration rate of Grimes in storage for the seasons of 1928-29 and 1929-30 is shown in table 8. Such factors as soil fertilization with sodium nitrate, conditions of immediate and deferred storage and the variation of fruit from one year to the other affected the respiration rate

at 30° or 36° F. very slightly. Temperature was the controlling factor. At higher temperatures other factors, such as maturity of the fruit and soil nutrient condition, in addition to the temperature factor, affected the respiration rate.

PERIODS OF RESPIRATORY ACTIVITY IN APPLES

A comparison of the respiration rate of Grimes stored at 50° F. for the two storage seasons of 1928-29 and 1929-30 shows that the apples entered storage in 1929 in a slightly more advanced stage of maturity as indicated by a slightly higher respiration rate. Data in table 6 shows that the peak of respiration in both seasons was reached within nearly the same length of time, 4 to 5 weeks after entering storage. By comparing figs. 4 and 5 it will be seen that the general trend of the curves for the 2 years is very similar, and in both cases the respiratory activity may be subdivided into 4 stages, as indicated previously.

Stage I. Period of accelerated ascent. Under a uniformly maintained temperature the rate of ascent and the height of respiration attained undoubtedly depended upon the amount of available respiratory reserves. This probably would be influenced by picking maturity.

Stage II. Period of rapid descent. From the peak finally attained the rate of respiration fell off suddenly. This stage is of short duration and extends through a period of about 2 to 3 weeks. The rapid decline was probably due to loss of certain available respiratory materials through respiration in Stage I.

Stage III. Period of gradual descent. The respiration rate in this stage was less rapid than in Stage II and extended over a period of approximately 6 weeks. This stage may be referred to as the period of stability in the respiratory process.

Stage IV. Period of senescence. The senescent period gives rise to an increased respiration rate. This was expected since complications arose as evidenced by the appearance of mealy breakdown, scald and apple mold.

Inasmuch as this curve may be divided into four distinct stages, there is indicated a depletion or exhaustion of specific respiratory materials. Chemical analyses have been conducted with comparable lots of apples which may help to explain the changes in respiratory material represented by the different stages.³

EFFECT OF NITRATE FERTILIZERS ON RESPIRATION

Grimes Golden from trees having previously received appli-

³ The results of these analyses have recently been published. Iowa State College Jour. of Sci. 9:95-114. 1934.

cations of nitrate fertilizers respired consistently more than did the check lots at a temperature of 50° F. At 36° and 30° F., temperature was the controlling factor, and previous environmental conditions did not express themselves in respiratory activity.

Figure 5 shows the rate of respiration from nitrated and check lots of apples stored immediately after picking, in storage temperatures of 50°, 36° and 30° F. Curve 1 was quite consistently higher than curve 2, especially during stages I, II and IV. As may be noted later, in the second part of this investigation (fig. 10), catalase activity at 50° F. was greater with nitrate-treated fruit than with check treatment lots.

DEFERRED STORAGE AND RESPIRATION IN APPLES

Apples comparable to those used for immediate storage investigations were used in the respiration studies on deferred storage. Grimes were delayed at 50° F. for 3 weeks before being placed in the respective lower temperatures of 36° and 30° F. Although the transfer from 50° to 36° or 30° F. did not occur at the very peak of respiration, it did come during the stage of accelerated ascent (stage I) while the apples were respiring at a high rate, as shown in fig. 6.

It has been pointed out that there was a consistent difference in respiratory activity between nitrated and check lots at 50° F. After transfer from 50° to 36° or 30° F. there was no

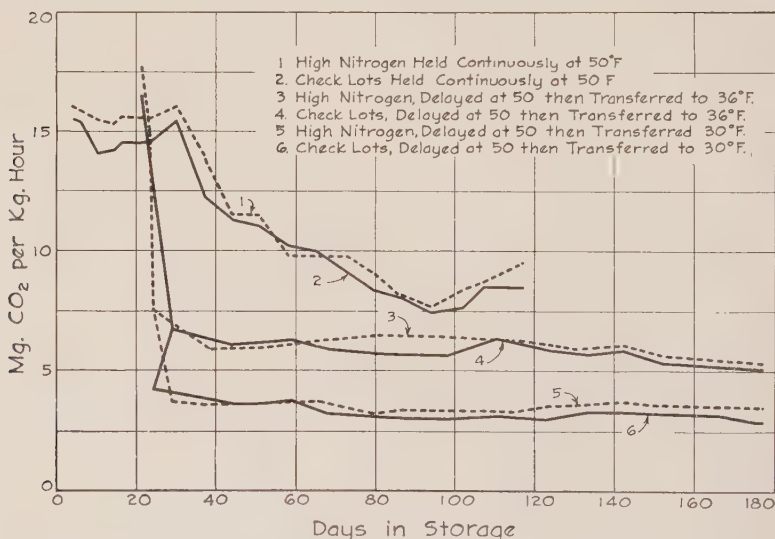


Fig. 6. Respiration rate of Grimes Golden stored at 50° F. continuously, and of fruit delayed at 50° and then transferred to lower temperatures of 36° and 30° F. Season 1929-30.

appreciable difference in respiratory activity between nitrated and check lots of apples. This substantiates the conclusions drawn in the case of immediately-stored fruit, that at temperatures of 36° or 30° F. factors other than temperature exert little influence upon the respiration rate. The data in tables 7 and 8 show how closely the respiration rates of fruit at 36° and 30° F. compare, whether the fruit was stored immediately or delayed, or whether the fruit was from nitrate-treated trees or from check lots.

THE RELATION OF RESPIRATORY ACTIVITY TO SOGGY BREAKDOWN

On March 22, 6 months after storing, apples in the respiration chambers were examined. Data in table 9 show the condition of the fruit at the end of the storage season. The percentage of shrinkage—with one exception—was below 2 percent (table 9). The two cases wherein the greatest shrinkage was found were with the fruit delayed at 50° for 3 weeks before storage at 36° F.

No soggy breakdown occurred when the fruit was stored immediately, but it developed when the apples had been transferred from 50°, or from a state of high respiratory activity, to 30° F. wherein the respiration rate was low. Collateral storage lots of fruit for 1929-30 confirmed these findings in the case of deferred storage and showed the following percentages of soggy breakdown: 3 weeks deferred storage at 50° before storage at 30° F. high nitrogen (nitrate 5-5-10) 43.7 percent; check lot 11.1 percent. No soggy breakdown developed in deferred storage fruit at 36° F. in either nitrated or check lots.

A comparison of these data with those of the fruit's condition at the end of the 1928-29 storage season (table 4) shows less soggy breakdown for 1929-30. In both seasons, soggy breakdown occurred only when fruit had been transferred from the higher temperature while respiratory activity was high to a lower temperature, under which condition the respiration rate was sharply reduced.

DISCUSSION

In respiration investigations extending throughout the two storage seasons of 1928-29 and 1929-30 three separate series of apples were used. These were subjected to various temperatures and storage conditions, and the rate of carbon dioxide evolution at weekly or 10-day intervals throughout the entire time the fruit was in storage, was used as a measure of respiratory activity.

That temperature is the controlling factor and exerts the

TABLE 7. THE RATE OF RESPIRATION OF GRIMES GOLDEN APPLES WHEN STORED AT 36°F. RESULTS FOR SEASONS 1928-29 AND 1929-30.*
Mg. CO₂ per kg. per hr.

Date of determination	Days in storage	1928-29		1929-30		1929-30	
		Nitrate 5-5		Nitrate 5-5-10		Check	
		Immediate storage	Deferred storage	Immediate storage	Deferred storage	Immediate storage	Deferred storage
Sept. 28	3	5.87					
Sept. 29	4					7.22	
Sept. 30	5	5.97		6.46			
Oct. 1	6	5.45					
Oct. 5	10			5.80		7.20	
Oct. 8	13	6.83				5.83	
Oct. 11	16						
Oct. 12	17			6.08			
Oct. 13	18			6.13			
Oct. 15	20	6.85		6.13			
Oct. 16	21				17.85		16.60
Oct. 18	23				14.98		14.30
Oct. 19	24		5.20		7.58		
Oct. 21	26		4.95				
Oct. 22	27	5.44					
Oct. 24	29						6.76
Oct. 26	31		6.39				
Oct. 27	32			6.02			
Oct. 28	33			5.86			
Oct. 29	34	5.32					
Oct. 31	36					6.13	
Nov. 1	37					6.02	
Nov. 2	38		6.19		5.91		
Nov. 5	41	4.47					
Nov. 8	44						6.09
Nov. 9	45		5.20				
Nov. 12	48	6.22					
Nov. 13	49			6.02			
Nov. 15	51					5.75	
Nov. 16	52		5.48		5.97		
Nov. 19	55	5.77					
Nov. 23	59		5.48				6.38
Nov. 26	62	5.77					
Nov. 29	65					6.29	
Nov. 30	66		5.26		6.24		
Dec. 2	68						5.92
Dec. 3	69	5.62					
Dec. 7	73		6.22				
Dec. 10	76	5.60		6.30			
Dec. 12	78					6.18	
Dec. 14	80		6.23		6.46		5.86
Dec. 17	83	6.00					5.79
Dec. 20	86						
Dec. 21	87		6.32				
Dec. 24	90	5.97		6.08			
Dec. 26	92					6.17	
Dec. 28	94		5.24		6.41		
Dec. 31	97	6.72					
Jan. 1	98						5.75
Jan. 4	101		5.24	6.52			
Jan. 7	104	7.42					
Jan. 8	105				6.32	6.13	
Jan. 11	108		5.38				
Jan. 14	111	7.41		6.30			6.28
Jan. 18	115		5.56		6.30	6.55	
Jan. 22	119	6.92					
Jan. 24	121			6.52			
Jan. 25	122		6.94			5.77	
Jan. 26	123				6.13		5.92
Feb. 1	129			6.52			
Feb. 2	130					7.57	
Feb. 3	131				5.91		
Feb. 5	133						5.75
Feb. 10	138			7.17			
Feb. 13	141					7.63	
Feb. 14	142				6.06		5.96
Feb. 20	148			6.46			
Feb. 23	151					6.55	
Feb. 24	152				5.64		5.38
Mch. 17	173			5.86			
Mch. 18	174					5.48	
Mch. 20	176				5.23		
Mch. 21	177						5.07

*Apples picked Sept. 25 in 1928, Sept. 26 in 1929.

TABLE 8. THE RATE OF RESPIRATION OF GRIMES GOLDEN APPLES WHEN
STORED AT 30°F. RESULTS FOR SEASONS 1928-29 AND 1929-30.*
Mg. CO₂ per kg. per hr.

Date of determination	Days in storage	1928-29		1929-30		1929-30	
		Nitrate 5-5		Nitrate 5-5-10		Check	
		Immediate storage	Deferred storage	Immediate storage	Deferred storage	Immediate storage	Deferred storage
Sept. 28	3	3.27					
Sept. 29	4						
Sept. 30	5					4.35	
Oct. 1	6	3.31		5.00			
Oct. 2	7					4.02	
Oct. 5	10			4.68			
Oct. 8	13	3.65				4.35	
Oct. 11	16						
Oct. 12	17			4.03			
Oct. 13	18			4.22			
Oct. 15	20	3.62					
Oct. 19	24		4.77				4.18
Oct. 21	26		4.18				
Oct. 22	27	3.62					
Oct. 24	29				3.82		
Oct. 26	31		3.29	4.08			
Oct. 27	32			3.70			
Oct. 28	33			3.65			
Oct. 29	34	3.62					
Oct. 31	36					4.24	
Nov. 1	37					4.52	
Nov. 2	38		3.16		3.65		
Nov. 5	41	3.11					
Nov. 9	45		3.61				3.67
Nov. 13	49	3.16		3.49			
Nov. 15	51					3.96	
Nov. 16	52		3.45		3.71		
Nov. 19	55	3.66					
Nov. 23	59		3.30				3.77
Nov. 24	60			3.43			
Nov. 26	62	3.50					
Nov. 29	65					4.07	
Nov. 30	66		3.45		3.76		
Dec. 2	68						3.24
Dec. 3	69	3.01					
Dec. 7	73		4.14				
Dec. 10	76	3.16		3.43			
Dec. 12	78					4.16	
Dec. 14	80		3.92		3.22		
Dec. 17	83	3.01					3.09
Dec. 20	86						
Dec. 21	87		4.19		3.38		
Dec. 24	90	3.01		3.70			
Dec. 26	92					4.02	
Dec. 28	94		3.30		3.38		
Dec. 31	97	4.37					
Jan. 1	98						3.03
Jan. 4	101		3.30	3.57			
Jan. 7	104	4.17					
Jan. 8	105				3.38	4.10	
Jan. 11	108		3.48				
Jan. 14	111	4.17		3.54			3.19
Jan. 18	115		3.60		3.33	4.13	
Jan. 21	118	4.87					
Jan. 24	121			4.03			
Jan. 25	122		2.55			4.16	
Jan. 26	123				3.60		3.09
Feb. 1	129			4.14			
Feb. 2	130				3.54	4.18	
Feb. 5	133						3.30
Feb. 10	138			4.08			
Feb. 13	141				3.82	4.47	
Feb. 14	142						3.30
Feb. 20	148			4.19			
Feb. 23	151					4.41	
Feb. 24	152				3.65		
Mch. 10	166						3.19
Mch. 16	172			4.19			
Mch. 18	174					4.02	
Mch. 20	176				3.52		2.98
Mch. 21	177						2.98

*Apples picked Sept. 25 in 1928; Sept. 26 in 1929.

TABLE 9. PERCENTAGE OF SHRINKAGE AND OF SOGGY BREAKDOWN OF THE FRUIT AT THE END OF THE STORAGE SEASON.
1929-30

Lot no.	Storage		Field treatment		Storage temp. F.	Number of apples	Original weight in grams	Percent-age shrink-age	Condition of fruit	
	Im-medi-ate	De-lay-ed	High nitro-gen	Check					Percent-age sound	Percent-age of soggy break-down
1	X		X		50	72	7,800			
2	X			X	50	70	8,210			
3	X		X		36	74	8,052	1.70	97	none
4	X			X	36	65	8,218	1.49	100	none
5		X	X		36	53	7,991	1.75	100	none
6		X		X	36	76	8,357	2.10	97	none
7	X		X		30	62	8,145	1.03	95	none
8	X			X	30	72	7,815	0.79	100	none
9		X	X		30	74	8,127	1.31	35	65
10		X		X	30	82	8,326	1.19	42	58

greatest influence on respiratory intensity is demonstrated by the preliminary investigations in which apples were subjected to alternating temperatures. Transfers from one temperature to the other were made three times during the season and the experiment which was repeated during the second year gave almost identical results. When the fruit was placed at 30° F. the respiration rate was reduced to a minimum and, each time the interchange was made from 30 to 50° and vice versa, approximately the same increased or reduced respiration rate was reached, depending on the temperature. It should be observed, however, that at the higher temperature the rate of respiration also appears to be influenced by respiratory reserves, and a general lowering of respiration is observed as the season advances. In other words, the stage of development or the maturity of the fruit expresses itself and affects the respiration rate.

Respiratory intensity as affected by soil nitrate, fertilization, time of picking, time of storing and storage temperatures was determined. Special attention was given to ascertain the role of respiration in the development of storage disorders of apples, with particular reference to the disorder known as soggy breakdown. That delayed storage materially increases the susceptibility of Grimes to soggy breakdown was established by Plagge and Maney (45). In a subsequent investigation Plagge (46) found an anomalous situation, in that soggy breakdown occurred when the fruit was delayed for 1 to 4 weeks before storage at the lower temperatures while if the delay was longer, from 6 to 12 weeks, soggy breakdown did not occur. The question arises, "Why does soggy breakdown occur under certain conditions of deferred storage and not when those conditions are aggravated?" Evidently the percentage of soggy break-

down is not to be correlated with the extent of the delay. Then, again, practically no soggy breakdown occurs when the fruit is held continuously at 50°, 30° or 36° F., which shows that it is not the result of either high temperatures or low temperatures alone.

The role which respiration plays in connection with the development of soggy breakdown advances in part an explanation as to the underlying cause and physiological condition which brings about disturbances in storage apples. When fruit was delayed at the higher temperature of 50° F. before transference to the lower cold storage temperatures of 30° or 36° F., it was transferred at a high rate of respiration to a condition which reduced respiratory activity to a minimum. Soggy breakdown developed when the transfer was made to lower temperatures while the apples were respiring at a high rate after 2 to 5 weeks' delay at 50° F. When the apples had passed the period of high respiratory activity, or during the period of lower activity (after 6 to 12 weeks' delay), the apples were transferred to the lower temperatures and soggy breakdown did not develop (stage III, fig. 5). Soggy breakdown occurred when Grimes were transferred during their period of greatest respiratory intensity, and, as has been emphasized, the highest percentage of breakdown occurred when the transfer was made at the peak of respiration -indicating that the greater the inhibition of respiration, the greater is the disturbance within the tissue of the apple as manifested by soggy breakdown.

In comparing respiratory intensity of Grimes stored at 50° F. for the seasons of 1928-29 and 1929-30 (table 6), the slightly higher respiration rate for the latter season may be attributed to a slightly more advanced stage of maturity or to the additional application of 10 pounds of nitrate fertilizer. The general trend of the curves for the 2 years is very similar (figs. 4, 5 and 6) and the form of the curve may be divided into four distinct stages or periods (fig. 5).

The keeping quality of Grimes, in relation to the effect of nitrogen treatment, was determined by measuring the respiratory intensity of apples from trees receiving nitrogenous fertilizers, and from trees having received no treatment. At 50° F. apples from the high nitrogen treatment respired consistently more than did check lots of apples. At 36° and 30° F., temperature was the controlling factor and previous nutrient or environmental conditions scarcely expressed themselves. Even in the case of deferred storage fruit, after being placed in the lower temperatures, there was no appreciable difference in the respiratory activity between nitrated and check lots. At 30° or 36° F., extraneous factors showed little effect and the respiration rate was very constant and fairly low whether the fruit was stored immediately or deferred, or whether the fruit was

from nitrate treated trees or from check lots (tables 7 and 8, figs. 5 and 6). Results of this investigation and collateral data from other experiments show a higher percentage of soggy breakdown in deferred storage fruit from the high nitrogen treatment than from the check lots of apples.

SUMMARY—PART II

1. In the cases of deferred storage fruit held at 30° and 36° F., and of immediately stored fruit held continuously at 50° F., catalase activity was greater at 30° F. than at the other two temperatures.

2. Respiratory intensity and catalase activity were not consistently correlated. At 50° F. catalase activity appeared to be associated with respiration, whereas at storage temperatures of 30° F. and 36° F. no parallelism occurred.

3. Grimes apples from nitrate-treated trees had greater catalase activity than similar fruit from untreated trees.

4. Catalase activity appears to be an indication of physiological conditions and disturbances within the tissue of the apple in storage. Under cold storage conditions, increase in catalase activity is a fairly accurate index to the approach of soggy breakdown.

5. Oxidase and catalase activity were independent of each other, and oxidase was not found to be significant as an indicator in denoting the development of soggy breakdown.

Part II. Catalase and Oxidase Activity

Only the literature on oxidase and catalase activity pertinent to the data presented here will be considered. The general topic has been thoroughly reviewed by Heinicke (23), Rhine (48), Ezell and Crist (14), Knott (32) and others.

HISTORICAL

Loew (34) believed that catalase was present in all cells of both plants and animals. Subsequent investigations by Reed (47), however, showed that in certain stages of development, the pineapple contains oxidase but not catalase. Appleman (1) found a striking correlation between catalase activity in the potato juice and respiratory activity in the tubers. On the other hand, he found no correlation between oxidase activity and respiratory intensity. Crocker and Harrington (12) confirmed to some extent the findings of Appleman. Overholser (42) reported an agreement between catalase and respiration in the fruit of the pear. The data indicated that the catalase activity of pears differed widely according to the variety. With the four varieties of pears tested, the two having lowest respira-

tory intensity were also lowest in catalase activity; and the two highest in respiratory intensity were highest in catalase activity.

On the other hand, Magness and Burroughs (37) found no direct association between catalase activity and respiratory intensity in the fruit of the apple. That catalase was not directly associated with temperature was shown by removing Baldwin apples from 65° storage to 32° F. storage. At the higher temperature high catalase activity had developed, but after 2 months in the lower temperature of 32° F. there was no apparent decrease in catalase activity.

Heinicke (23) reported no consistent relation between respiratory intensity and catalase activity. But the same author pointed out that this does not indicate that catalase activity is without metabolic significance. In early germination of seeds, as pointed out by Rhine (48), catalase and respiration not only are not parallel but are for a time running counter to each other, one decreasing while the other increases. Drain (13) found that varieties of apples differ in catalase activity and that respiration rate and catalase activity are not closely correlated.

Overholser (43) found that catalase activity in pears was greater at 20° and 28° than at 0° C., when the pears were transferred from 0° and held at 20° or 28° C. for 3 to 14 days. Storage at 0° and 30° C. tended to result in a gradual increase during the total storage period of 22 days. After 6 months' storage at 0° C. catalase was greater than when harvested.

In experimenting with low temperatures, Carrick (11) found that extreme freezing of the McIntosh apple, wherein most of the cells were killed, markedly reduced catalase activity.

Heinicke (21) stated that the higher the nitrogen content, or the higher the proportion of nitrogen to carbohydrates, the greater the catalase activity. These results were substantiated by Auchter (2), who found a greater catalase activity in leaves on the nitrate-fertilized sides of trees. The nitrogen treatment apparently stimulated the catalase activity of the apple even when chemical analysis failed to show increased nitrogen in the leaves.

In 1927 Ezell and Crist (14) reported a negative correlation between growth or size of lettuce and spinach, and catalase and oxidase activity. Results reported by Haber (17) in respect to decreased catalase activity with increases in growth of leaves and yield of fruit in the tomato were in accord with results of Ezell and Crist. Haber (17) found that catalase activity of the tomato fruit was lowest in very green fruit, greater in the mature green stage, and less in the ripe fruit.

Results of Gourley and Hopkins (16) in 1929 are in accord with those of Heinicke (22, 23) and Auchter (2). From their

studies one may conclude that nitrates produce larger, less highly colored fruit, higher water content, greater amount and percentage of nitrogen, and increased catalase activity with a close correlation between catalase activity and nitrogen percentage in the fruit.

Knott (32) states that when metabolism is shifted so that the plant changes from a vegetative to a reproductive type of growth, catalase activity decreases. Evidence was obtained by Overholser (42) that catalase activity was highest in young pears and tended to decrease markedly as maturity advanced. This greater catalase activity may be correlated with the greater vegetative activity of the young fruits, compared with the lower vegetative activity of the mature fruits. To the same effect, Heinicke (24, 25) points out that catalase activity was greater during the year of the smaller crop, and that the heavier the crop, the lower the activity.

No correlation between growth, yield and oxidase activity was reported by Haber (17). Reed (47) found catalase and oxidase activity to be independent of each other, and this has been substantiated by Haber (17), Appleman (1), Ezell and Crist (14) and Drain (13).

METHODS AND APPARATUS FOR THE DETERMINATION OF CATALASE AND OXIDASE ACTIVITY

During the storage season of 1929-30, catalase and oxidase activity were determined on Grimes Golden apples which were comparable in respect to field treatment and storage conditions to the apples used for respiration studies.

Determinations were made periodically throughout the storage season. For measuring catalase activity 20 apples were used as representative of each storage treatment (35). Catalase and oxidase activity were determined on five composite samples of each treatment, and the averages of the readings were used to indicate the activity for each treatment. Every precaution was taken to obtain a uniform sample and a uniform suspension of the macerated tissue. No trouble was encountered in checking closely duplicate samples or samples of the same treatment.

The method employed for taking the sample was similar to that used by Carrick (11). A cork borer, 1 centimeter in diameter, was used. The boring was made transversely through the center of the fruit. Each end of the cylinder of tissue was cut off and the core region removed, so that the sample contained largely parenchyma tissue from the cortex portion of the apple. A 4-gram sample was weighed immediately and mixed with an equal weight of calcium carbonate (3), and 2 cc. of distilled water. The sample was then ground in a mortar until a creamy

mixture was obtained. This suspension was entirely removed from the mortar and brought to a volume of 20 cubic centimeters. The solutions were kept at the various specific storage temperatures in tightly stoppered test tubes until used. Both catalase and oxidase determinations were made within 24 hours from the time of the preparation of the sample.

Standard procedure for the determination of catalase activity, similar to that used by Haber (17), was followed; with Harvey's (20) modification of the Bunzel (6) oxidase apparatus. Parts of the same samples used for catalase determinations were used for the measurements of oxidase activity. The procedure was the same as that used by Haber (17).⁴

⁴For a more complete account of the procedure followed, the reader is referred to the author's original thesis on file in the library of Iowa State College, Ames, Iowa.

EXPERIMENTAL

RELATION OF CATALASE AND OXIDASE ACTIVITY TO TEMPERATURE, NITRATE FERTILIZATION AND RESPIRATION

Determinations of catalase and oxidase activity on Grimes Golden apples were made periodically throughout the storage season. Data in tables 10 and 11 show the catalase and oxidase

TABLE 10. CATALASE ACTIVITY OF GRIMES GOLDEN APPLES UNDER DIFFERENT STORAGE TEMPERATURES, AND OTHER CONDITIONS. EACH RECORD IS THE AVERAGE OF 5 DETERMINATIONS OF THE VOLUME OF OXYGEN IN CC. EVOLVED IN 5 MINUTES AT 68°F. SAMPLE OF TISSUE TAKEN FROM COMPOSITE SAMPLES OF 20 APPLES.

Date of determination	Storage temperatures									
	50°F.		36°F.				30°F.			
	Immediate storage		Immediate storage		3 weeks delayed storage		Immediate storage		3 weeks delayed storage	
	High N. c.c.	Check c.c.	High N. c.c.	Check c.c.	High N. c.c.	Check c.c.	High N. c.c.	Check c.c.	High N. c.c.	Check c.c.
Pre-commercial picking										
9-6-29	2.5	2.9								
9-17-29	3.5	3.3								
Commercial picking										
9-28-29	9.1	6.1	9.1	6.1	9.1	6.1	9.1	6.1	9.1	6.1
10-17-29	11.1	9.6	8.5	5.6	11.1	9.6	5.9	5.6	11.1	9.6
11-6-29	10.7	9.2	8.3	6.4	10.9	8.2	6.8	5.8	8.4	7.9
11-29-29	5.1	4.3	8.2	7.2	10.8	7.9	9.8	9.2	17.2	11.8
12-15-29	4.1	4.3	8.1	8.4	14.5	11.3	11.1	9.1	16.4	11.4
1-5-30	3.3	2.9	9.4	8.9	10.2	6.8	9.6	8.7	14.0	10.2
2-10-30	2.6	2.2	5.9	5.9	7.9	6.5	7.6	7.6	10.9	10.8
3-18-30	2.5	2.5	4.9	3.9	5.5	4.3	7.3	7.2	5.2	6.0

TABLE 11. OXIDASE ACTIVITY OF GRIMES GOLDEN APPLES UNDER DIFFERENT STORAGE TEMPERATURES, AND OTHER CONDITIONS. EACH RECORD IS THE AVERAGE OF 5 DETERMINATIONS OF OXIDASE IN CC. OF MERCURY DISPLACED AT THE END OF 60 MINUTES AT 68°F. COMPOSITE SAMPLES OF TISSUE FROM 20 APPLES.

Date of determination	Storage temperatures									
	50°F.		36°F.				30°F.			
	Immediate storage		Immediate storage		3 weeks delayed storage		Immediate storage		3 weeks delayed storage	
	High N.	Check	High N.	Check	High N.	Check	High N.	Check	High N.	Check
Pre-commercial picking										
9-6-29	1.2	0.8								
9-17-29	1.3	1.3								
Commercial picking										
9-28-29	1.3	1.6	1.3	1.6	1.3	1.6	1.3	1.6	1.3	1.6
10-17-29	1.4	1.7	1.2	1.2	1.4	1.7	1.1	1.1	1.4	1.7
11-6-29	1.2	0.8	1.3	1.2	1.1	0.9	1.2	1.0	0.9	0.9
11-29-29	0.9	1.0	0.9	0.8	1.1	1.0	1.1	1.0	1.1	0.9
1-5-30	1.0	0.9	0.9	0.9	1.1	1.1	1.1	1.1	1.0	0.9
2-10-30	1.0	0.9	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
3-18-30	0.9	0.9	0.9	0.75	0.95	0.95	0.9	0.8	0.9	1.0

activity of Grimes picked prior to the time of the commercial picking as well as those picked at the commercial picking time and stored at the various specified temperatures. Catalase and oxidase activity at 50° F. increased as the fruit approached prime condition, then decreased markedly as the storage season advanced and the fruit deteriorated. Catalase activity was more constant throughout the storage season when the apples were placed immediately in the storage temperatures of 30° and 36° F. When the apples were delayed for 3 weeks at 50° F. before storage at 30° and 36° F., this constancy was greatly affected and the catalase activity stimulated. Additional stimulatory effect may be noted with high nitrogen treatment. The highest catalase activity occurred just prior to the appearance of soggy breakdown at the storage temperature of 30° F. High catalase activity was observed with delayed storage fruit at 36° F., although the activity was not as great nor was the increase so abrupt as at 30° F. A small amount of soggy breakdown appeared with delayed storage fruit at 36° F., although it was present in an incipient form. High catalase activity therefore seems to be closely associated with a subsequent appearance of soggy breakdown.

Catalase and oxidase determinations were made on the broken-down fruit tissue which had developed soggy breakdown.

TABLE 12. CATALASE AND OXIDASE ACTIVITY FROM FOUR GRAMS OF
BROKEN-DOWN TISSUE OF THE APPLES IN WHICH SOGGY
BREAKDOWN HAD DEVELOPED.

March 28

Number of sample	Catalase. cc. of water displaced in 5 minutes	Oxidase. cc. of mer- cury displaced at the end of 60 minutes
1	0.0	0.3
2	0.0	0.3
3	0.2	0.4
4	0.15	0.5
5	0.20	0.4
6	0.0	0.3
7	0.15	0.4
8	0.0	0.3
9	0.05	0.2
10	0.1	0.4

An almost immeasurable small amount of oxygen was evolved from this tissue as is shown in table 12.

On the whole, oxidase determinations were consistently unrevealing insofar as its relation to soggy breakdown was concerned.

THE EFFECT OF STORAGE TEMPERATURE ON CATALASE ACTIVITY

Catalase activity is independent of, and unrelated to, temperature. As shown in figs. 7 and 8, catalase activity is higher with delayed storage fruit than immediately stored fruit and

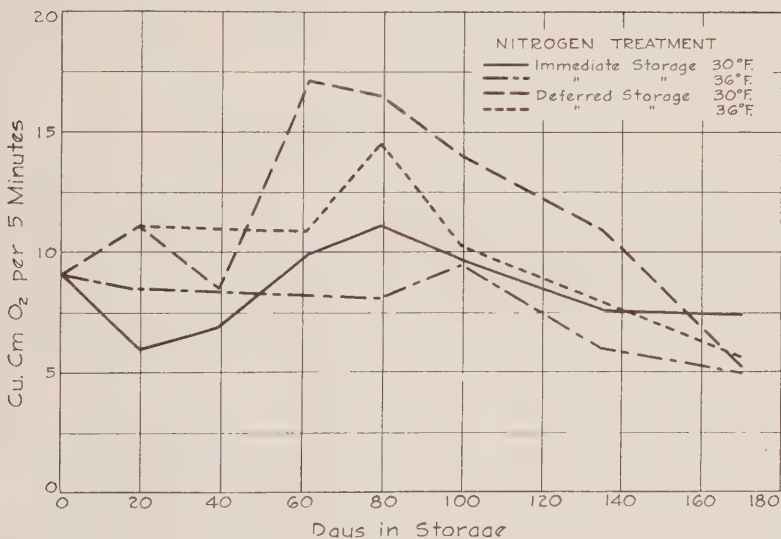


Fig. 7. Effect of storage temperature with immediate and deferred fruit on catalase activity of apples from nitrate-fertilized trees.

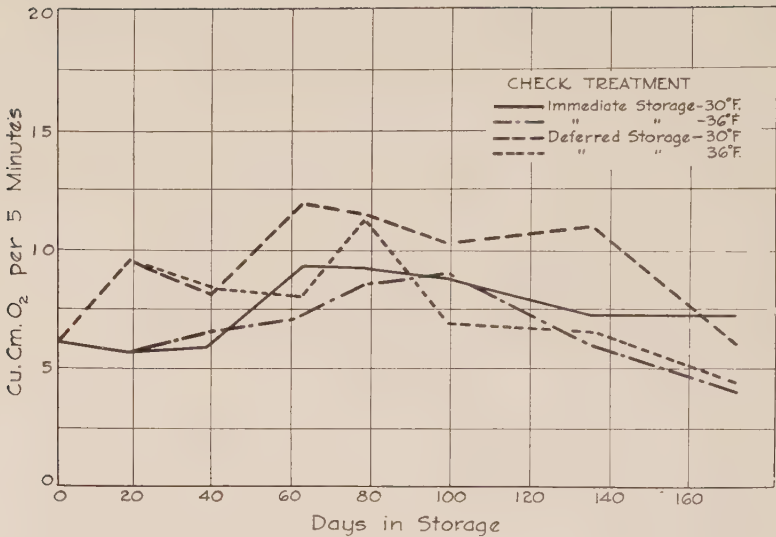


Fig. 8. The effect of storage temperature with immediate and deferred storage on catalase activity of apples from check lots.

higher and more irregular at 30° F. than at 36° F., with apples from both nitrogen fertilization and check lots.

It has been pointed out that soggy breakdown developed when Grimes were delayed at higher temperatures and transferred to 30° F. Figs. 7 and 8 show that the catalase activity increased considerably prior to the appearance of this storage disorder. This evidence bears out Heinicke's (25) view that catalase is a sensitive indicator of metabolism.

THE EFFECT OF NITRATE FERTILIZATION ON CATALASE ACTIVITY

Catalase activity was closely correlated with nitrogen fertilization, as is shown in figs. 9 and 10. With immediate storage fruit at 30° and 36° F., the nitrogen treated apples were consistently higher in catalase activity than the check lot apples as is shown in fig. 9. In the case of delayed storage apples the catalase activity was considerably higher in fruit receiving nitrate treatment than in those of the check lot which had not received nitrate. This point is depicted in fig. 9. At the higher storage temperature of 50° F. the same relationship existed between nitrate fertilization and catalase activity, fruit of the nitrate treatment showing a consistently higher catalase activity than that of the check lot. This relationship is shown in fig. 10.

Under these various storage conditions and temperatures, whether immediately stored or delayed 3 weeks at 50° F. before

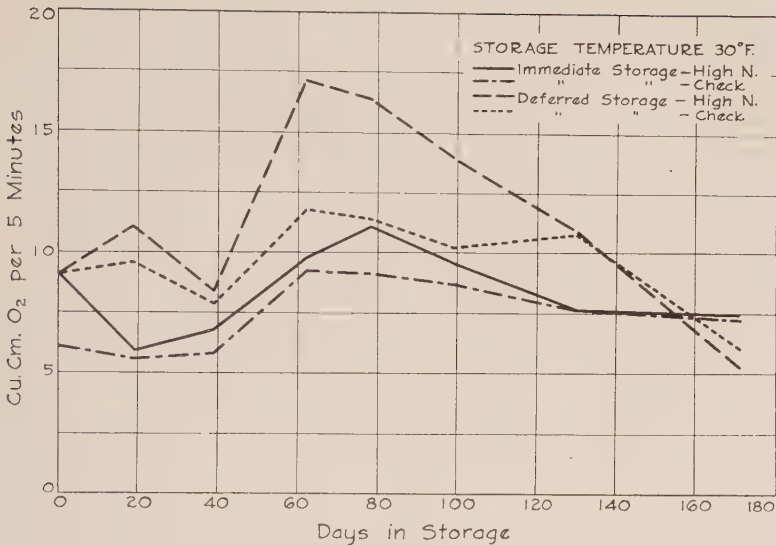


Fig. 9. The effect of nitrogenous fertilizers on catalase activity in fruit stored at 30° F. with immediate and deferred storage treatment.

being subjected to the storage temperatures of 36° and 30° F. or whether maintained at 50° F. throughout storage, there was consistently greater catalase activity in apples from nitrate-treated trees.

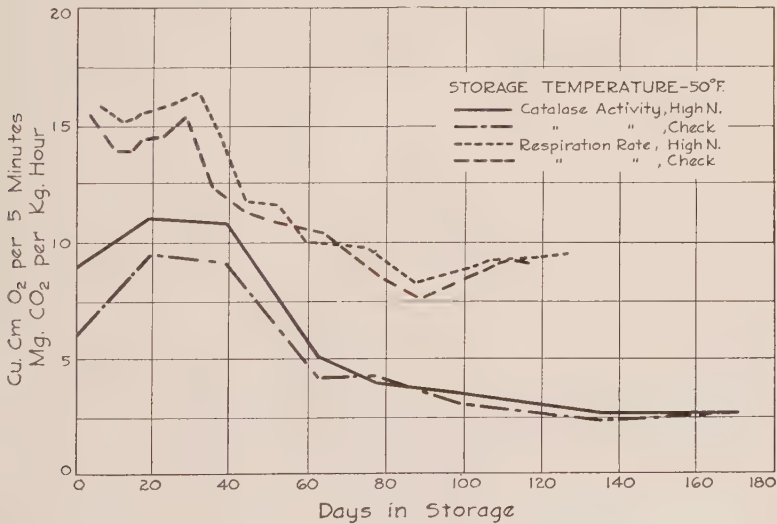


Fig. 10. The relation between catalase activity and respiratory intensity of fruit held continuously at 50° F.

RELATIONSHIP OF CATALASE ACTIVITY TO RESPIRATORY INTENSITY

Catalase has been considered a respiratory enzyme by many workers. The data and curves presented here show no correlation between catalase activity and respiratory intensity at the lower temperatures, although at 50° F. catalase activity is apparently related to respiration (fig. 10). Fig. 11, representing both immediate and delayed storage fruit held at 30° F., shows that there was no corresponding increase in respiration rate with the rise of catalase activity. This was especially noticeable in deferred storage fruit, where catalase activity fluctuated independently of the respiratory intensity. Lack of parallelism between catalase activity and respiratory intensity holds true for fruit stored at 30° and 36° F. and whether determined from fruit of nitrate-treated trees or check lots from non-nitrated trees.

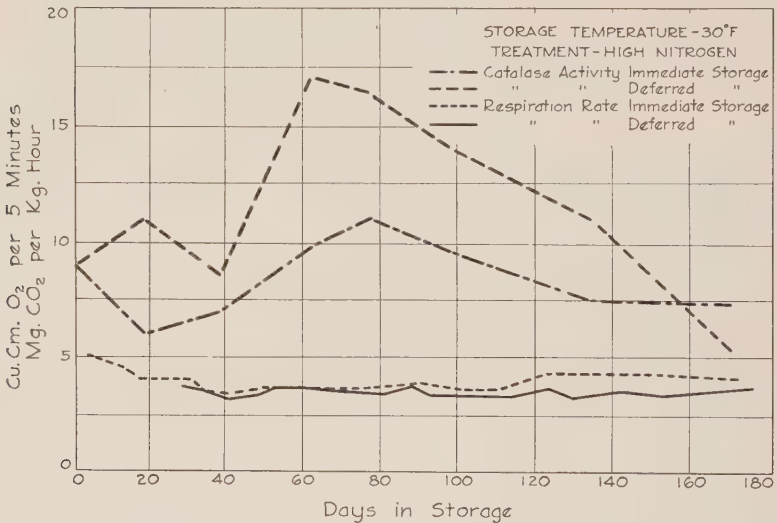


Fig. 11. Catalase activity and respiratory intensity of fruit from nitrogen treatment stored at 30° F.

DISCUSSION

Grimes Golden apples comparable to those in the respiration studies were used in making determinations of catalase and oxidase activity. The object of this investigation was to determine whether these physiological activities are associated, and also what role each plays in the development of storage disorders.

Apples from plots receiving nitrate fertilization and check plots were subjected to the same variations of storage temperature and conditions of immediate and deferred storage. Curves comparing catalase activity and respiratory intensity have been presented. Since little conclusive evidence was gained in regard to oxidase, this discussion deals largely with catalase activity.

Catalase activity was correlated with nitrogen fertilization. With both immediate and deferred storage at 30° and 36° F. and continuous storage at 50° F. fruit from nitrate-treated plots was consistently higher in catalase activity. These results are comparable with the work of Heinicke (21) and Gourley and Hopkins (16).

In reviewing the literature on these two enzymes, some workers have considered catalase and oxidase as respiratory enzymes. Data and results of the present investigation show no correlation with respiration at the lower temperatures, 30° and 36° F. This lack of correlation between respiratory and catalase activity has been reported by Rhine (48), Drain (13), Heinicke (23) and Magness and Burroughs (37). In the present study, however, catalase activity at 50° F., while not closely correlated, appeared to be related to respiration.

In dealing with deferred storage fruit after placement under low temperature and prior to the appearance of soggy breakdown, high catalase activity was indicated, with no corresponding increase in respiratory intensity (fig. 11). Catalase activity under these conditions appears to be more sensitive than respiration as an indicator of the approach of this storage disorder. A noticeable increase in catalase activity early in the storage season is a good indication of soggy breakdown susceptibility. The highest catalase activity with deferred storage fruit at 30° F. occurs just prior to and during the early stages of the development of soggy breakdown. With deferred storage fruit at 36° F., even an incipient form of soggy breakdown is indicated by catalase activity, although the disease does not actually appear until considerably later.

Deferred storage of Grimes Golden apples is contributory to storage disorders, such as soggy and mealy breakdown. Catalase activity serves as an indicator of physiological behavior, indicating disturbances within the tissue of the apple.

LITERATURE CITED

- (1) Appleman, Chas. O. Relations of catalase and oxidases to respiration in plants. Md. Agr. Exp. Sta., Bul. 191. 1915.
- (2) Auchter, E. C. Is there normally a cross transfer of foods, water and mineral in wood plants? Md. Agr. Exp. Sta., Bul. 257. 1923.
- (3) Becking, L. G. M.B., and Hampton, H. C. Measurement of the catalytic power of catalase. Amer. Jour. Bot., 7:261-274. 1920.
- (4) Bigelow, W. D., Gore, H. C., and Howard, B. J. Studies on apples. U. S. Bur. of Chem., Bul. 95. 1905.
- (5) Brooks, Charles, Cooley, J. S., and Fisher, D. F. Apple scald. Jour. Agr. Res., 16:195-217. 1919.
- (6) Bunzel, H. H. A simplified and inexpensive oxidase apparatus. Jour. Biol. Chem., 17:409-411. 1914.
- (7) Burroughs, A. M. Changes in respiration rate of ripening apples. Proc. Amer. Soc. Hort. Sci., 19:225-234. 1922.
- (8) Burroughs, A. M. Studies in apple storage. In storage investigations, 1921-1922. The Marble Laboratory, Inc., 101-137, Canton, Pa.
- (9) Carrick, D. B. The respiration of apples at low non-freezing temperatures and while frozen. Proc. Amer. Soc. Hort. Sci., 23:277-285. 1926.
- (10) Carrick, D. B. The effect of freezing on the respiration of the apple. N. Y. (Cornell) Agr. Exp. Sta., Memoir 110. 1928.
- (11) Carrick, D. B. The effects of freezing on the catalase activity of apple fruits. N. Y. (Cornell) Agr. Exp. Sta., Memoir 122. 1929.
- (12) Crocker, William and Harrington, George T. Catalase and oxidase content of seeds in relation to their dormancy, age, vitality and respiration. Jour. Agr. Res., 15:137-174. 1918.
- (13) Drain, Brooks D. Temperature and respiration enzymes of apples. Bot. Gaz., 82:183-194. 1926.
- (14) Ezell, B. D. and Crist, J. W. Effect of certain nutrient conditions on activity of catalase and oxidase. Mich. Agr. Exp. Sta., Tech. Bul. 78. 1927.
- (15) Gore, H. C. Studies on fruit respiration. U. S. Bur. Chem., Bul. 142. 1911.
- (16) Gourley, J. H. and Hopkins, E. F. Some relations of nitrogen to keeping quality of fruit. Proc. Amer. Soc. Hort. Sci., 26:167-173. 1929.
- (17) Haber, E. S. Catalase and oxidase of the tomato as influenced by the soil reaction. Iowa State College Jour. Sci., 3:29-39. 1928.
- (18) Harding, P. L. Respiration studies of Grimes Golden apples under various controlled temperatures. Proc. Amer. Soc. Hort. Sci., 26:319-324. 1929.
- (19) Harding, P. L., Maney, T. J. and Plagge, H. H. Apparatus for the determination of carbon dioxide in the respiration of apples. Science, 70:125. 1929.
- (20) Harvey, R. B. An apparatus for measuring oxidase and catalase activity. Jour. Gen. Physiol., 2:253-254. 1920.
- (21) Heinicke, A. J. Catalase activity as an indicator of the nutritive conditions of fruit tree tissue. Proc. Amer. Soc. Hort. Sci., 19:209-214. 1922.
- (22) Heinicke, A. J. Factors influencing catalase activity in apple leaf tissue. N. Y. (Cornell) Agr. Exp. Sta., Memoir 62. 1923.

- (23) Heinicke, A. J. Catalase activity in dormant apple-twigs; its relation to the condition of the tissue, respiration and other factors. N. Y. (Cornell) Agr. Exp. Sta., Memoir 74. 1924.
- (24) Heinicke, A. J. The influence of fruiting on the catalase activity of the bark of apple trees. Proc. Amer. Soc. Hort. Sci., 23: 263-269. 1926.
- (25) Heinicke, A. J. Seasonal variation in the nutrient condition of apple trees as indicated by catalase activity. Proc. Amer. Soc. Hort. Sci., 25:234-239. 1928.
- (26) Hill, George R., Jr. Respiration of fruits and growing plant tissues in certain gases, with reference to ventilation and fruit storage. N. Y. (Cornell) Agr. Exp. Sta., Bul. 330. 1913.
- (27) Kidd, Franklin and West, Cyril. Brown heart—A functional disease of apples and pears. Gt. Brit. Dept. Sci. and Indus. Res., Food Investigation Board. Special Rpt. 12. 1923.
- (28) Kidd, Franklin and West, Cyril. The storage life of apples in relation to respiratory activity and chemical composition. Gt. Brit. Dept. Sci. and Indus. Res., Food Investigation Board. Rpt. 1925-1926:37-57. 1927.
- (29) Kidd, Franklin and West, Cyril. Low-temperature injury in the cool storage of fruits and vegetables. Gt. Brit. Dept. Sci. and Indus. Res., Food Investigation Board. Rpt. 1931:122-127. 1932.
- (30) Kidd, Franklin and West, Cyril. Two types of storage internal breakdown in apples. Gt. Brit. Dept. Sci. and Indus. Res., Food Investigation Board. Rpt. 1927:42-43. 1928.
- (31) Kidd, Franklin and West, Cyril. Fruit and vegetables. Gt. Brit. Dept. Sci. and Indus. Res., Food Investigation Board. Rpt. 1928: Section B. 1929.
- (32) Knott, J. E. Catalase in relation to growth and to other changes in plant tissue. N. Y. (Cornell) Agr. Exp. Sta., Memoir 106. 1927.
- (33) Kostychev, S. Plant respiration, by Charles Lyon. P. Blakiston's Son and Co., Philadelphia. 1927.
- (34) Loew, Oscar. Catalase, a new enzyme of general occurrence, with special reference to the tobacco plant. U. S. Dept. Agr. Rpt. 68:1-47. 1901.
- (35) Loomis, W. E. Methods for analysis of horticultural material. Proc. Amer. Soc. Hort. Sci., 23:285-293. 1926.
- (36) Magness, J. R. Composition of the gases in the intercellular spaces of apples. Bot. Gaz., 70:308-316. 1920.
- (37) Magness, J. R. and Burroughs, A. M. Studies in apple storage. In storage investigations, 1921-1922, The Marble Laboratory, Inc., 17-98, Canton, Pa. 1923.
- (38) Magness, J. R. and Diehl, H. C. Physiological studies on apples in storage. Jour. Agr. Res., 27:1-38. 1924.
- (39) Magness, J. R., Diehl, H. C. and Haller, N. H. The ripening, storage, and handling of apples. U. S. Dept. Agr., Bul. 1406: 51-63. 1926.
- (40) Maney, T. J., Harding, P. L. and Plagge, H. H. A new type of respiration chamber. Science, 70:44. 1929.
- (41) Morse, F. W. The respiration of apples and its relation to their keeping. N. H. Agr. Exp. Sta., Bul. 135. 1908.
- (42) Overholser, E. L. A study of the catalase and oxidase activities of fruits of pear varieties. Unpublished thesis. Cornell University. 1926.
- (43) Overholser, E. L. A study of the catalase of the fruits of pear varieties. Amer. Jour. Bot., 15:285-306. 1928.

- (44) Palladin, V. I. Palladin's plant physiology. Edited with notes and additions by Burton E. Livingston. P. Blakiston's Son & Co., Philadelphia. Third Edition. 1922.
- (45) Plagge, H. H. and Maney, T. J. Soggy breakdown of apples and its control by storage temperatures. Iowa Agr. Exp. Sta., Res. Bul. 115. 1928.
- (46) Plagge, H. H. A study of soggy breakdown and some related functional diseases of apples. Proc. Amer. Soc. Hort. Sci., 26: 315-318. 1929.
- (47) Reed, G. B. The relation between oxidase and catalase in plant tissue. Bot. Gaz., 62:409-412. 1916.
- (48) Rhine, Louisa E. Divergence of catalase and respiration in germination. Bot. Gaz., 78:46-67. 1924.
- (49) Smith, A. J. and Student, F. S. Brown heart in Australian apple shipments. Gt. Brit. Dept. Sci. and Indus. Res., Food investigation Board. Special Rpt. 22. 1925.
- (50) Truog, E. Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrimetric method. Jour. of Indus. and Engin. Chem., 7:1045-1049. 1915.

